



US005624803A

United States Patent [19]

Noonberg et al.

[11] **Patent Number:** 5,624,803[45] **Date of Patent:** Apr. 29, 1997

[54] **IN VIVO OLIGONUCLEOTIDE GENERATOR, AND METHODS OF TESTING THE BINDING AFFINITY OF TRIPLEX FORMING OLIGONUCLEOTIDES DERIVED THEREFROM**

[75] Inventors: **Sarah B. Noonberg**, Berkeley; **C. Anthony Hunt**, San Francisco, both of Calif.

[73] Assignee: **The Regents of the University of California**, Oakland, Calif.

[21] Appl. No.: **324,001**

[22] Filed: **Oct. 13, 1994**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 138,666, Oct. 14, 1993, abandoned.

[51] **Int. Cl.**⁶ **C12Q 1/68**; **C12N 15/63**;
C12N 15/11

[52] **U.S. Cl.** **435/6**; 435/91.1; 435/320.1;
536/24.1

[58] **Field of Search** 435/6, 172.3, 320.1;
536/23.1, 24.1, 91.1

[56] **References Cited****U.S. PATENT DOCUMENTS**

5,190,931	3/1993	Inouye	435/91.32
5,272,065	12/1993	Inouye et al.	435/91.1
5,316,930	5/1994	Loesch-Fries et al.	435/172.3

FOREIGN PATENT DOCUMENTS

0601585	6/1994	European Pat. Off.
95/06744	3/1995	WIPO
95/06718	3/1995	WIPO

OTHER PUBLICATIONS

Case et al., "The unusual stability of the IS10 anti-sense RNA is critical for its function and is determined by the structure of its stem-domain" *EMBO J.* (1989) 8:4297-4305.

Chrissey et al., "Antisense technology: Principles and prospects for therapeutic development" *BioPharm* (1991) pp. 36-42.

Cooney et al., "Site-specific oligonucleotide binding represses transcription of the human c-myc gene in vitro" *Science* (1988) 241:456-459.

Das et al., "Upstream regulatory elements are necessary and sufficient for transcription of a U6 RNA gene by RNA polymerase III" *EMBO J.* (1988) 7:503-512.

Durland et al., "Binding of triple helix forming oligonucleotides to sites in gene promoters" *Biochem.* (1991) 30:9246-9255.

Duval-Valentin et al., "Specific inhibition of transcription by triple helix-forming oligonucleotides" *Proc. Natl. Acad. Sci. USA* (1992) 89:504-508.

Hannon et al., "Multiple cis-acting elements are required for RNA polymerase III transcription of the gene encoding H1 RNA, the RNA component of human RNase P" *J. Biol. Chem.* (1991) 266:22796-22799.

Hélène, "The anti-gene strategy: control of gene expression by triplex-forming-oligonucleotides" *Anti-Cancer Drug Design* (1991) 6:569-584.

Izant, "Chimeric antisense RNAs" *Gene Regulation: Biology of Antisense RNA and DNA* (1992) Erickson, R.P. et al., eds., Raven Press, New York, pp. 183-195.

Jennings et al., "Inhibition of SV40 replicon function by engineered antisense RNA transcribed by RNA polymerase III" *EMBO J.* (1987) 6:3043-3047.

Junker et al., "Reduction in replication of the human immunodeficiency virus type 1 in human cell lines by polymerase III-driven transcription of chimeric tRNA-antisense RNA genes" *Antisense Res. & Develop.* (1994) 4:165-172.

Kunkel et al., "U6 small nuclear RNA is transcribed by RNA polymerase III" *Proc. Natl. Acad. USA* (1986) 83:8575-8579.

Kunkel et al., "Transcription of a human U6 small nuclear RNA gene in vivo withstands deletion of intragenic sequences but not for an upstream TATATA box" *Nucleic Acids Res.* (1989) 17:7371-7379.

Marshallsay et al., "Characterization of the U3 and U6 snRNA genes from wheat: U3snRNA genes in monocot plants are transcribed by RNA polymerase III" *Plant Mol. Biol.* (1992) 19:973-983.

Moffat, "Making sense of antisense" *Science* (1991) 253:510-511.

Murphy et al., "The in vitro transcription of the 7SK RNA gene by RNA polymerase III is dependent only on the presence of an upstream promoter" *Cell* (1987) 51:81-87.

Noonberg et al., "Detection of triplex-forming RNA oligonucleotides by triplex blotting" *BioTechniques* (1994) 16:1070-1072.

Noonberg et al., "In vivo generation of highly abundant sequence-specific oligonucleotides for antisense and triplex gene regulation" *Nucleic Acids Res.* (1994) 22:2830-2836.

Sullenger et al., "Expression of chimeric tRNA-driven antisense transcripts renders NIH 3T3 cells highly resistant to Moloney murine leukemia virus replication" *Mol. & Cell. Biol.* (1990) 10:6512-6523.

Terns et al., "Multiple cis-acting signals for export of pre-U1 snRNA from the nucleus" *Genes & Development* (1993) 7:1898-1908.

Williard et al., "Paradoxical production of target protein using antisense RNA expression vectors" *Gene* (1994) 149:21-24.

Willis, "RNA polymerase III. Genes, factors and transcriptional specificity" *Eur. J. Biochem.* (1993) 212:1-11.

Yuan et al., "5' flanking sequences of human MRP7-2 RNA gene are required and sufficient for the transcription by RNA polymerase III" *Biochim. Biophys. Acta* (1991) 1089:33-39.

Lyamichev et al., *Nucleic Acids Res.* 16: 2165 (1988).

Primary Examiner—James Martinell

[57] **ABSTRACT**

The present invention encompasses improved methods and materials for the delivering of antisense, triplex, and/or ribozyme oligonucleotides intracellularly, and RNA polymerase III-based constructs termed "oligonucleotide generators" to accomplish the delivery of oligonucleotides. Also encompassed by the present invention are methods for screening oligonucleotide sequences that are candidates for triplex formation.